View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

International Journal of Infectious Diseases 17 (2013) e1176–e1181

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Olive baboons: a non-human primate model for testing dengue virus type 2 replication

Iris Valdés [a,1], Lázaro Gil [a,1], Jorge Castro [a], Damián Odoyo [b], Rikoi Hitler [b], Elephas Munene [b], Yaremis Romero [a], Lucy Ochola [b], Karelia Cosme [a], Thomas Kariuki [b], Gerardo Guillén [a], Lisset Hermida [a,*]

[a] Center for Genetic Engineering and Biotechnology (CIGB), Avenue 31, PO Box 6162, Havana 6, 10 600, Cuba
[b] Institute of Primate Research (IPR), National Museum of Kenya, Nairobi, Kenya

ARTICLE INFO

Article history:
Received 20 May 2013
Received in revised form 23 July 2013
Accepted 7 August 2013

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Dengue
Baboons
African green monkeys
Non-human primates
Vaccine
Viremia

SUMMARY

Objective: This study evaluated the use of a non-human primate, the olive baboon (*Papio anubis*), as a model of dengue infection. Olive baboons closely resemble humans genetically and physiologically and have been used extensively for assessing novel vaccine formulations.
Methods: Two doses of dengue virus type 2 (DENV-2) were tested in baboons: 10^3 and 10^4 pfu. Similarly, African green monkeys received the same quantity of virus and acted as positive controls.
Results: Following exposure, high levels of viremia were detected in both animal species. There was a trend to detect more days of viremia and more homogeneous viral titers in animals receiving the low viral dose. In addition, baboons infected with the virus generally exhibited positive virus isolation 1 day later than African green monkeys. Humoral responses consisting of antiviral and neutralizing antibodies were detected in all animals after infection.
Conclusions: We conclude that baboons provide an alternative non-human primate species for experimental DENV-2 infection and we recommend their use for further tests of vaccines, administering the lowest dose assayed: 10^3 pfu.
© 2013 International Society for Infectious Diseases. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Dengue is the most prevalent arthropod-borne viral disease in humans, with more than two billion people living in risk areas, and it is estimated that there are nine million symptomatic cases and 500 000 severe episodes of dengue worldwide each year.[1]

The dengue illness is transmitted by the bite of mosquitoes of the genus *Aedes*.[2] It is caused by four antigenically related but distinct dengue virus (DENV) serotypes (DENV-1 to DENV-4), which belong to the family *Flaviviridae*, genus *Flavivirus*.[2] Infection with DENV results in either asymptomatic or symptomatic disease, ranging from classical dengue fever (DF) to more severe cases of dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS).[3] Despite the high incidence of this disease, there is currently no vaccine to prevent the spread of dengue disease or reduce its incidence worldwide. One of the issues that have hampered the development of the vaccine is the lack of a suitable animal model.[4]

Several species have been used to evaluate vaccine candidates in preclinical studies. Although non-human primates (NHP) do not develop the classical signs of dengue disease, this model is valuable for studying immune responses after infection with the virus and for evaluating several vaccine candidates.[5] After virus infection, susceptible animals develop viremia and humoral and cellular responses, but do not exhibit the classical clinical signs observed in humans.[5] The more common species employed have been rhesus (*Macaca mulatta*),[6–11] cynomolgus (*Macaca fascicularis*),[12] and New World monkeys.[13,14]

In previous studies, our group demonstrated that the African green monkey (*Chlorocebus aethiops sabaeus*) species is able to provide a potential model for preclinical assessment of novel vaccines.[15,16] In these animals, the mean viremia duration with DENV-2 was 1.6 to 5.6 days depending on the viral dose used.[15] On the other hand, when NHP were immunized with the vaccine candidate, this viremia period was reduced.[17]

This present study proposed to evaluate the olive baboon (*Papio anubis*) as a suitable animal model for DENV-2 infection. These NHPs belong to the group of Old World monkeys and are widely distributed in many parts of Africa, including northern, southern-central, and eastern Africa.[18] Their body size makes them

* Corresponding author. Tel.: +53 7 271 6022; fax: +53 7 271 4764.
E-mail address: lisset.hermida@cigb.edu.cu (L. Hermida).
[1] These authors contributed equally to the work.

1201-9712/$36.00 – see front matter © 2013 International Society for Infectious Diseases. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ijid.2013.08.007

particularly well-suited to a number of clinical procedures in preclinical studies,[19] and for the extraction of sufficient blood volumes during the period of viremia in order to test the parameters required for dengue vaccine assessment.

In the present study, two doses of DENV-2 were tested in baboons: 10^3 and 10^4 plaque-forming units (pfu). Similarly, African green monkeys received the same quantity of virus and acted as positive controls. It was possible to isolate the virus from all animals included in the study, and the animals developed a robust humoral immune response after virus infection. These results demonstrate the potential value of baboons as a model for dengue vaccine testing.

2. Materials and methods

2.1. Animals

Healthy young adult (2–5 kg) African green monkeys (*Chlorocebus aethiops sabaeus*) and healthy young adult (16–22 kg) olive baboons (*Papio anubis*), provided by the Institute of Primate Research (IPR, Nairobi, Kenya), were used in this study. All animals were screened for previous exposure to DENV by ELISA and plaque reduction neutralization test (PRNT). Animals were considered naive when antigen-specific antibodies were undetectable by ELISA (titer <1:50) or PRNT (titer <1:10). NHPs were maintained in accordance with the Kenyan guidelines for the care and use of laboratory animals, and all experimental procedures were approved by independent scientific and ethics committees at the IPR, Nairobi, Kenya.

2.2. Viruses

Infection was performed with a low-passage DENV-2 strain SB8553 (Asian genotype), isolated in 2002 from a human case of DF in Sibu, Malaysia (kindly provided by Dr M.J. Cardosa, University Sarawak, Malaysia). Aliquots of the infection virus were stored frozen at −80 °C at a titer of 2×10^6 pfu/ml. The standard strain New Guinea C of DENV-2 was used as sucrose–acetone antigen[20] for immunoassay tests.

2.3. Maintenance and clinical assessment

Animals were maintained throughout the study in individual cages that permitted conduct patterns to be evaluated according to the species, size, age, and sex. They were fed with commercial monkey chow, supplemented with fruits and vegetables. Water was available ad libitum. Infected baboons and African green monkeys were subjected to daily clinical inspections of lymphatic ganglia, skin, and respiratory, digestive, and nervous systems. The rectal temperature and body weight of all animals was assessed daily by the veterinary staff. Clinical biochemistry testing was performed on animals on day 0 and day 30 after infection, to avoid additional stress to the animals. These animals showed similar behavior to healthy animals; they did not show any signs of pain or distress at any time during the study. All results were reported and records maintained in accordance with IPR and Kenyan guidelines for animal use in research. The IPR follows international guidelines for the use of animals in biomedical research, as it is a World Health Organization Collaborating Center and has statutory registration with the NIH Office of Laboratory Animal Welfare, in addition to local and wide recognition in Africa as a Center of Excellence in preclinical studies.

2.4. Virus infection and detection of viremia

Six monkeys of each species were ranked by weight, age, and sex and then randomly divided into two groups of three animals each. Prior to each procedure, animals were anaesthetized with an intramuscular injection. African green monkeys were anaesthetized using ketamine hydrochloride, 10 mg/kg body weight. Baboons, due to their weight, were anaesthetized with a mixture of ketamine hydrochloride at 10 mg/kg and xylazine hydrochloride at 2 mg/kg body weight. Each animal was then infected subcutaneously with 1 ml (0.5 ml in each upper arm) of virus at doses of 10^3 or 10^4 pfu. The inoculums of 10^3 or 10^4 pfu were obtained by diluting a preparation of 2×10^6 pfu/ml in RPMI-1640 medium immediately prior to infection.

Starting on day 0, 4 ml of blood was collected daily from each animal for 11 days to detect viremia. For serological studies, the same volumes of blood were taken at day 30 post challenge. Animals were observed for 30 min after each inoculation for immediate local and systemic reactions and daily for the next 10 days.

Serum from clotted blood was stored at −70 °C until viremia was analyzed. The presence of virus in serum was determined by inoculating 140 μl of serum diluted 1:10 onto Vero cell monolayers grown in 25-cm^2 flasks. Fresh supplemented RPMI-1640 medium (5% heat inactivated fetal bovine serum, 2 mM L-glutamine, and 100 U of penicillin–streptomycin) was added. The cultures were incubated for 4 h at 37 °C in a 5% CO_2 atmosphere. Next, 2.5 ml of 3% medium-viscosity carboxymethylcellulose was added. Plates were incubated at 37 °C for 6 days in a 5% CO_2 atmosphere. To visualize the viral plaques, the monolayer was dyed with naphthol blue black solution (naphthol blue black 0.1%, sodium acetate 0.2 M, acetic acid 6%). Viremia was determined using the $\log_{10}$-transformed plaque counts detected on Vero cells ($\log_{10}$ pfu/ml).

2.5. Analysis of the antibody response

The anti-DENV IgG antibodies were monitored by ELISA. Briefly, flat-bottomed 96-well plates (Costar, USA) were coated with the monoclonal antibody 4G2, which recognizes the *Flavivirus* E protein,[21] in coating buffer (0.16% Na_2CO_3, 0.29% $NaHCO_3$, pH 9.5). Three washes with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (v:v) (PBS-T; Merck, Germany) were completed after each step. Plates were blocked with 2% bovine serum albumin (BSA), and then incubated overnight at 4 °C with a saturating concentration of DENV antigen and mock antigen in separate wells. Serially diluted samples from serum were incubated for 1 h at 37 °C with either the DENV or the mock antigen. Anti-monkey IgG-peroxidase conjugate (Sigma, USA) was added and the plates were incubated for 1 h at 37 °C. After washing, 0.04% substrate solution (*o*-phenylenediamine in buffer 2% Na_2HPO_4, 1% citric acid, and 30% H_2O_2, pH 5.0) was added. The plates were kept for 30 min at 25 °C and the reaction was stopped with 12.5% H_2SO_4. Absorbance was read at 492 nm in a microplate reader (SensIdent Scan; Merck, Germany). Titers were defined as the dilution of serum giving twice the absorbance value of the negative control serum.

Antibody functionality was measured by neutralization of DENV-2 (strain SB8553) infectivity by a PRNT on Vero cell culture as described previously.[22] The assay assessed four dilutions to calculate the 50% plaque end-point serum titers. The neutralizing antibody titer was identified as the highest serum dilution that reduced the number of virus plaques by 50% and was calculated by using a four-point linear regression method. The monoclonal antibody 4G2 was used as positive control, which recognizes the *Flavivirus* E protein.[21]

2.6. Statistical analysis

Data from the humoral antiviral immune response were assessed using the non-parametric Kruskal–Wallis test and Dunn

multiple comparison test. Humoral neutralizing response data were analyzed by the non-parametric Mann–Whitney test. In all cases, GraphPad Prism version 5.00 for Windows was employed (GraphPad Software; http://www.graphpad.com).

3. Results

3.1. Physical and clinical parameters

Body temperature in all monkeys was normal throughout the study (data not shown).

A reduction in body weight during the period of daily blood sampling was evident for baboons (Figure 1A). The average decrease in this group was around 1 kg of the total weight during the first 10 days. The degree of weight loss observed was expected given the frequent chemical sedation undergone by the animals. At the end of the study, the animals had completely recovered their weight. In contrast, there was no change in the body weight of any of the six African green monkeys during the blood sampling period (Figure 1B).

Animals were subjected to daily clinical inspection and no signs of physiological disturbance were detected during the study period. All monkeys showed behavioral patterns characteristic of healthy animals. Viremia was monitored from day 0 to day 10 of infection, and the immunogenicity was assessed on day 0 and day 30.

3.2. Replication of DENV-2 in baboons and African green monkeys

The objective of this study was to compare the replicative capacity of two viral doses of DENV-2 (10^3 and 10^4 pfu) in two different species of monkey. Serum samples were collected daily from the baboons and African green monkeys infected with DENV-2 (strain SB8553) for the detection of viremia. As shown in Table 1, animals from both groups were able to replicate DENV-2 and become viremic after the infection.

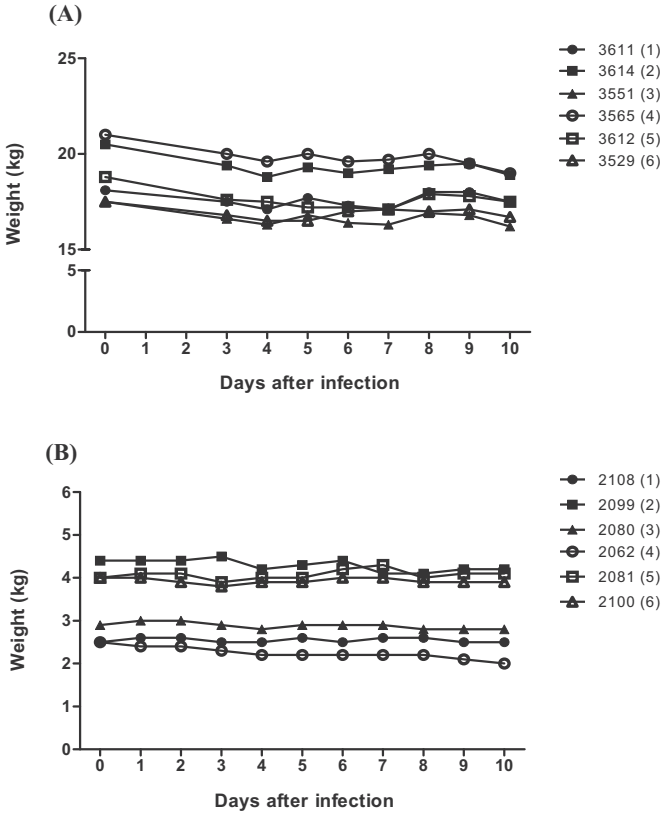

Figure 1. Changes in body weight of the animals infected with dengue virus type 2. (A) Baboons. (B) African green monkeys. Closed symbols represent the animals infected with 10^3 pfu and open symbols represent the animals infected with 10^4 pfu.

Typical viremia behavior was observed in all baboons, similar to previous observations describing the use of NHPs as a model for dengue infection. However, in this experiment the baboons showed more homogeneous behavior compared to the African green monkeys (Table 1).

In general, all animals developed viremia, which started at day 2–3. The viremia lasted 4 to 5 days (mean duration of 4–4.3 days) in both species after infection with a high viral dose (10^4 pfu). In baboons and African green monkeys that received the lower viral dose, viremia lasted 4.6 and 5.7 days, respectively. Intermittent viremia was only detected in one animal, an African green monkey that belonged to the group infected with 10^4 pfu (monkey 2080) (Table 1).

For practical and ethical reasons, a small number of animals was used in this study, hence the results are descriptive; only general trends were analyzed and are discussed. As shown in the Table 1, regardless of the viral dose used, a higher viremia was observed in baboons than in African green monkeys (1.9 $\log_{10}$ pfu/ml to 2.6 $\log_{10}$ pfu/ml vs. 1.5 $\log_{10}$ pfu/ml to 1.8 $\log_{10}$ pfu/ml, for baboons vs. African green monkeys, respectively).

3.3. Immune responses

All animals infected with DENV-2 developed a humoral immune response against the homologous virus measured by ELISA and PRNT. Total IgG titers against the four serotypes were also measured by ELISA at day 30 post infection (Table 2). For both groups, African green monkeys and baboons, an antiviral antibody response was developed against four serotypes, however the highest antiviral antibody titers were observed against the homologous serotype ($p < 0.05$).

When comparing the viral dose in the two animal species, the monkeys that received the low dose elicited similar anti-DENV-2 titers (5333.3 in baboons and 6666.6 in African green monkeys), and the same occurred in the animals receiving the highest dose (9333.3 in baboons and 13333.3 in African green monkeys). Similarly, both species developed similar anti-DENV-2 titers (Table 2) regardless of the viral dose tested (GMT 7333.3 for baboons and GMT 10000 for African green monkeys), without statistical differences between them ($p = 0.3061$, by non-parametric Mann–Whitney test).

The neutralizing response was evaluated at day 30 post infection. In contrast to the antiviral response, neutralizing titers showed statistical differences between the species ($p < 0.05$). Baboons elicited lower titers of neutralizing antibodies (GMT 471.6) compared to those developed by the African green monkeys at the same time-point (GMT 1066.7), whatever the viral inoculum dose (Table 3).

4. Discussion

The NHP model has been proposed as a suitable model for dengue infection. Although these animals are not able to reproduce all clinical symptoms of dengue disease seen in humans, they have been used to evaluate the replication and the immune response to vaccines.[4,5] They are, however, limited by availability and expense, as well as by safety and logistical considerations arising from animal size, temperament, and the potential for zoonotic disease transmission.[23]

In this study we evaluated the possibility of using the olive baboon as an alternative model for DENV-2 infection. Baboons are

Table 1
Viremia after infection with dengue virus type 2 (DENV-2) in African green monkeys and baboons

	Viral dose	Monkey	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Total days	Mean of viremia (log_{10} pfu/ml)	Mean of viremic days
Baboons	DENV-2	3611(1)	0	0	0	1.7[a]	2.3	2.6	2.7	0.9	0	0	0	5	2.0	4
	10^4 pfu	3614(2)	0	0	0	1.9	2.7	2.6	2.3	0	0	0	0	4	2.4	
		3551(3)	0	0	0	0	2.5	2. 8	2.5	0	0	0	0	3	2.6	
	DENV-2	3565(4)	0	0	0	0.9	1.8	2.2	2.7	2.4	1.7	0	0	6	2.0	4.6
	10^3 pfu	3612(5)	0	0	0	0	1.7	2.4	2.6	0	0	0	0	3	2.2	
		3529(6)	0	0	0	1.4	2.0	2.2	2.2	1.9	0	0	0	5	1.9	
African green monkeys	DENV-2	2108(1)	0	0	1.3	1.3	1.9	1.8	1.4	0	0	0	0	5	1.5	4.3
	10^4 pfu	2099(2)	0	0	0	0.9	1.9	2.2	2.0	0	0	0	0	4	1.7	
		2080(3)	0	0	1.8	1.8	1.5	0	1.2	0	0	0	0	4	1.6	
	DENV-2	2062(4)	0	0	0	1.2	2.1	2.6	1.5	0	0	0	0	4	1.8	5.7
	10^3 pfu	2081(5)	0	0	0	0	1.2	2.2	2.3	2.3	1.5	1.2	0	6	1.8	
		2100(6)	0	0	0.9	1.5	2.2	2.0	1.5	1.2	1.2	0	0	7	1.5	

[a] Viral detection was assessed by direct plaque formation on Vero cells.

robust animals, allowing us to obtain sufficient blood sample volumes, mainly during the viremic period. They are abundant in Africa, and unlike African green monkeys, are not susceptible to simian immunodeficiency virus infection. In addition, baboons are more closely related to macaques than *Chorocebus* species,[24] and have also been used as models for other viral infections such as West Nile virus, herpesvirus, and Rift Valley fever virus.[25–27]

DENV-2 infection in baboons did not change the temperature pattern during the viremic period. Similar results have been reported for other NHP species.[5,15] Conversely, body weight during the blood sampling period decreased more noticeably in baboons than in African green monkeys. These differences could be explained by the chemical agents used for the sedation procedure in each species. While African green monkeys received only ketamine, baboons, due to their large size, required a mixture of the more aggressive anesthetic ketamine–xylazine to reach a proper sedation status.[28] In addition, there was a delay in food intake by baboons after the administration of anesthesia, as observed during daily inspections; therefore, we speculate that this fact could be responsible for the weight loss recorded in this species.

We also demonstrated that baboons become infected with strain SB8553 in a similar manner to the African green monkeys in terms of viremia duration and homogeneity. In particular, olive baboons not only showed a viremia similar to that of African green monkeys, but also the viremia started at day 3 post infection, which is an important issue in studies for dengue vaccine testing. This initial delay in virus replication could allow an adequate stimulation of the immunological memory induced by a vaccine to control the viral replication. It has been reported that the intrinsic incubation period in humans after inoculation of the virus by the mosquito bite is 4.5 to 7 days, even exceeding 10 days in some cases.[29]

The ability of olive baboons to replicate DENV makes them a suitable NHP model to study dengue infection, thus offering an alternative to the use of macaques. The mean viremia titer (2.2 log_{10} pfu/ml) and duration (4.3 days) are comparable to those described for *Macaca mulatta*[6–11] and *Macaca fascicularis*,[12] and are even higher than those described for Aotus monkeys[13,14] (Table 4).

The present study had a second intrinsic objective: to study the effect of the viral dose inoculated on the magnitude of the viremia in the two species tested. Previously, we reported that high viral doses (10^6 pfu) in African green monkeys did not increase the viremia level detected in infected animals. In this case, an early short-term viremia was induced, as compared with the viremia detected in monkeys receiving the low viral dose (10^4 pfu).[15] Accordingly, in the present work, there was a trend to detect more days of viremia and more homogeneous viral titers in animals receiving the low viral dose, for both animal species. Nevertheless, the observed difference between the viremia

Table 2
IgG antiviral antibodies against the four serotypes from sera of baboons or African green monkeys measured at day 30 after infection with 10^4 or 10^3 pfu[a]

	Viral dose	Monkey	DENV-1[c]	DENV-2[b]	DENV-3[c]	DENV-4[bc]
Baboons	DENV-2 (10^4 pfu)	3611(1)	1600	8000	1600	1600
		3614(2)	200	4000	400	800
		3551(3)	3200	16 000	3200	12 800
		GMT	**1666.6**	**9333.3**	**1733.3**	**5066.6**
	DENV-2 (10^3 pfu)	3565(4)	1600	8000	800	1600
		3612(5)	800	4000	400	800
		3529(6)	400	4000	200	1600
		GMT	**933.3**	**5333.3**	**466.6**	**1333.3**
African green monkeys	DENV-2 (10^4 pfu)	2108(1)	800	8000	800	1600
		2099(2)	400	16 000	800	1600
		2080(3)	800	16 000	1600	6400
		GMT	**666.6**	**13 333.3**	**1066.6**	**3200**
	DENV-2 (10^3 pfu)	2062(4)	200	4000	200	400
		2081(5)	400	8000	200	800
		2100(6)	800	8000	400	1600
		GMT	**466.6**	**6666.6**	**266.6**	**933.3**

[a] Statistical analysis was performed by one-way analysis of variance, using the non-parametric Kruskal–Wallis and Dunn multiple comparison test. Different letters (b, c) indicate statistical differences ($p < 0.05$).

Table 3
Neutralizing antibodies against DENV-2 in sera from baboons or African green monkeys measured at day 30 after infection. Neutralizing antibody titers are the highest serum dilution which resulted in a 50% reduction in the number of plaques produced by DENV-2 (SB8553)[a]

	Viral dose	Monkey	Neutralizing antibodies titers
Baboons[b]	DENV-2 (10^4 pfu)	3611(1)	640
		3614(2)	367
		3551(3)	530
		GMT	**512.3**
	DENV-2 (10^3 pfu)	3565(4)	640
		3612(5)	331
		3529(6)	322
		GMT	**431**
African green monkeys[c]	DENV-2 (10^4 pfu)	2108(1)	1280
		2099(2)	640
		2080(3)	1280
		GMT	**1066.7**
	DENV-2 (10^3 pfu)	2062(4)	640
		2081(5)	1280
		2100(6)	1280
		GMT	**1066.7**

[a] Statistical analysis was performed using the non-parametric Mann–Whitney test. Different letters (b, c) indicate statistical differences ($p < 0.05$).

Table 4
Summary of the viremia data from different non-human primate species used for dengue virus type 2 replication

Monkey species	Days of viremia	Onset of viremia	Mean of viremia ($\log_{10}$ pfu/ml)	References
Macaca mulatta	5.7	2.5	2.1	6–11
Macaca fascicularis	5.3	2.0	-	12
Aotus nancymaae	1.7	2.2	-	13,14
Chlorocebus aethiops	5.5	2.7	1.6	15–17
Papio anubis	4.3	3.3	2.2	This article

patterns for the two doses tested was not as evident as that detected in the previously published work.[15] A possible explanation could be related to the difference between the doses tested in the present work (1 $\log_{10}$) compared to that in the previous study (2 $\log_{10}$).

The humoral immune response was also evaluated in this study. The antiviral antibody response, measured by ELISA, was higher to the homologous serotype and lower and cross-reactive to the rest of the serotypes. Similar behavior has been observed in previous studies conducted in macaques and African green monkeys.[6,15] In addition, there was no evident difference between the titers obtained in the two species and even between the doses assayed.

Conversely, there were evident differences in terms of neutralizing antibodies. Olive baboons elicited lower titers compared to those developed by African green monkeys, whatever the viral dose assayed. This difference was not expected since the viremia pattern in the two species was almost the same. One possible explanation could be a difference in the kinetics of the neutralizing antibody response in the two species, therefore day 30 is not necessarily the best time point to compare the species. To confirm this hypothesis a kinetic study should be carried out for the two species.

In conclusion, this study is the first to report olive baboons as a suitable animal model for DENV-2 infection, adding them to the list of the few NHP species suitable for dengue vaccine testing. Studies on the other dengue serotypes will be carried out. In addition, we propose to evaluate the cellular immune response and the duration of the immunity induced after infection, and consequently its protective role upon reinfection with the same serotype, in further studies.

Acknowledgements

The authors thank James Ndungú and Prisilla Kimiti from the IPR, Kenya, for their technical support in the manipulations of the animals. The authors also thank Dr Ricardo Silva and Dr Harold Curiel for their critical reading of and useful comments on the revision of the manuscript. This investigation received financial support from the Cuban Program for Dengue Vaccine Development.

Conflict of interest: No conflict of interest to declare.

References

1. World Health Organization. Dengue and dengue haemorrhagic fever. 2011. http://www.who.int/mediacentre/factsheets/fs117/en/2009 (accessed 5 Sept 2011).
2. Gubler DJ. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends Microbiol* 2002;**10**:100–3.
3. Halstead SB. Dengue. *Curr Opin Infect Dis* 2002;**15**:471–6.
4. Bente DA, Rico-Hesse R. Models of dengue virus infection. *Drug Discov Today Dis Models* 2006;**3**:97–103.
5. Halstead SB, Shotwell H, Casals J. Studies on the pathogenesis of dengue infection in monkeys. I. Clinical laboratory responses to primary infection. *J Infect Dis* 1973;**128**:7–14.
6. Guirakhoo F, Pugachev K, Zhang Z, Myers G, Levenbook I, Draper K, et al. Safety and efficacy of chimeric yellow fever–dengue virus tetravalent vaccine formulations in nonhuman primates. *J Virol* 2004;**78**:4761–75.
7. Deubel V, Kinney RM, Esposito JJ, Cropp CB, Vorndam AV, Monath TP, et al. Dengue 2 virus envelope protein expressed by a recombinant vaccinia virus fails to protect monkeys against dengue. *J Gen Virol* 1988;**69**(Pt 8):1921–9.
8. Putnak JR, Coller BA, Voss G, Vaughn DW, Clements D, Peters I, et al. An evaluation of dengue type-2 inactivated, recombinant subunit, and live-attenuated vaccine candidates in the rhesus macaque model. *Vaccine* 2005;**23**:4442–52.
9. Simmons M, Porter KR, Hayes CG, Vaughn DW, Putnak R. Characterization of antibody responses to combinations of a dengue virus type 2 DNA vaccine and two dengue virus type 2 protein vaccines in rhesus macaques. *J Virol* 2006;**80**:9577–85.
10. Sun W, Nisalak A, Gettayacamin M, Eckels KH, Putnak JR, Vaughn DW, et al. Protection of Rhesus monkeys against dengue virus challenge after tetravalent live attenuated dengue virus vaccination. *J Infect Dis* 2006;**193**:1658–65.
11. Clements DE, Coller BA, Lieberman MM, Ogata S, Wang G, Harada KE, et al. Development of a recombinant tetravalent dengue virus vaccine: immunogenicity and efficacy studies in mice and monkeys. *Vaccine* 2010;**28**:2705–15.
12. Bernardo L, Izquierdo A, Alvarez M, Rosario D, Prado I, Lopez C, et al. Immunogenicity and protective efficacy of a recombinant fusion protein containing the domain III of the dengue 1 envelope protein in non-human primates. *Antiviral Res* 2008;**80**:194–9.
13. Kaufman BM, Summers PL, Dubois DR, Eckels KH. Monoclonal antibodies against dengue 2 virus E-glycoprotein protect mice against lethal dengue infection. *Am J Trop Med Hyg* 1987;**36**:427–34.

14. Kochel TJ, Watts DM, Gozalo AS, Ewing DF, Porter KR, Russell KL. Cross-serotype neutralization of dengue virus in *Aotus nancymae* monkeys. *J Infect Dis* 2005;**191**: 1000–4.
15. Martin J, Hermida L, Castro J, Lazo L, Martinez R, Gil L, et al. Viremia and antibody response in green monkeys (*Chlorocebus aethiops sabaeus*) infected with dengue virus type 2: a potential model for vaccine testing. *Microbiol Immunol* 2009;**53**:216–23.
16. Martin J, Hermida L, Castro J, Romero Y, Cardosa J, Guillen G. Viremia and the magnitude of the immune response upon infection of green monkeys with dengue virus type 2 are strain-dependent. *Curr Microbiol* 2009;**59**: 579–83.
17. Valdes I, Hermida L, Martin J, Menendez T, Gil L, Lazo L, et al. Immunological evaluation in nonhuman primates of formulations based on the chimeric protein P64k-domain III of dengue 2 and two components of *Neisseria meningitidis*. *Vaccine* 2009;**27**:995–1001.
18. Kennedy RC, Shearer MH, Hildebrand W. Nonhuman primate models to evaluate vaccine safety and immunogenicity. *Vaccine* 1997;**15**:903–8.
19. Perry DL, Bollinger L, White GL. The baboon (*Papio spp.*) as a model of human Ebola virus infection. *Viruses* 2012;**4**:2400–16.
20. Churdboonchart V, Bhamarapravati N, Peampramprecha S, Sirinavin S. Antibodies against dengue viral proteins in primary and secondary dengue hemorrhagic fever. *Am J Trop Med Hyg* 1991;**44**:481–93.
21. Kaufman BM, Summers PL, Dubois DR, Eckels KH. Monoclonal antibodies against dengue 2 virus E-glycoprotein protect mice against lethal dengue infection. *Am J Trop Med Hyg* 1987;**36**:427–34.
22. Roehrig JT, Hombach J, Barrett AD. Guidelines for plaque-reduction neutralization testing of human antibodies to dengue viruses. *Viral Immunol* 2008;**21**:123–32.
23. Carlsson HE, Schapiro SJ, Farah I, Hau J. Use of primates in research: a global overview. *Am J Primatol* 2004;**63**:225–37.
24. Goodman M. The genomic record of humankind's evolutionary roots. *Am J Hum Genet* 1999;**64**:31–9.
25. Wolf RF, Papin JF, Hines-Boykin R, Chavez-Suarez M, White GL, Sakalian M, et al. Baboon model for West Nile virus infection and vaccine evaluation. *Virology* 2006;**355**:44–51.
26. Whitby D, Stossel A, Gamache C, Papin J, Bosch M, Smith A, et al. Novel Kaposi's sarcoma-associated herpesvirus homolog in baboons. *J Virol* 2003;**77**:8159–65.
27. Papin JF, Verardi PH, Jones LA, Monge-Navarro F, Brault AC, Holbrook MR, et al. Recombinant Rift Valley fever vaccines induce protective levels of antibody in baboons and resistance to lethal challenge in mice. *Proc Natl Acad Sci U S A* 2011;**108**:14926–31.
28. Green CJ, Knight J, Precious S, Simpkin S. Ketamine alone and combined with diazepam or xylazine in laboratory animals: a 10 year experience. *Lab Anim* 1981;**15**:163–70.
29. Halstead S. Dengue, First ed., London: Imperial College Press; 2008.